EXPRESSION OF A TICK TOXIN FOR THE DEVELOPMENT OF A CANINE VACCINE

BY

JESSICA MAN-WEI CHUNG

Thesis submitted in fulfillment of the requirements of the degree of Master in the Faculty of Science, University of Technology, Sydney

September 2001

DECLARATION

CERTIFICATE OF AUTHORSHIP / ORIGINALITY

I certify that this thesis has not previously been submitted for a degree nor has it been submitted as part of requirements for a degree except as fully acknowledged within the text.

I also certify that the thesis has been written by me. Any help that I have received in my research work and the preparation of the thesis itself has been acknowledged. In addition, I certify that all information sources and literature used are indicated in the thesis.

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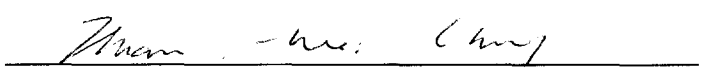

ACKNOWLEGEMENTS

I sincerely thank my supervisor Associate Professor Kevin Broady for his continued support and dedication to this project, who also taught me diverse approaches to problem solving and for reviewing this thesis patiently.

This project would be a mission impossible without the assistance of Slavica Masina, Carmen Lopresti, and Darren Jones regarding bacterial expression and monoclonal antibody techniques and Matthew Padula for the chromatographic work and reviewing the introduction.

I appreciate for friendship and technical assistance the present members of Immunobiology Unit, especially Joyce To, Andre Choo, Roie Gorman and Susan Lemke. Thank you all for making the time that I spent in the laboratory more enjoyable.

Finally, I thank my family and friends (Lyn Chen, Christine Lu, Sakura Narasimhan, and Fifin Infan) for their encouragement and faith in me.

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ABBREVIATIONS

$(Abu)_8$ AahII	*Androctonus australis hector* toxin analog
AaHIT	*Androctonus australis hector* insect toxin
AaIT	*Androctonus australis hector* insect selective toxin
AcNPV	*Autographa californica Nuclear Polyhedorsis* Virus
BCIP	5-bromo-4-chloro-3-indolyl phosphate
BM	*Boophilus microplus*
BmK	*Buthus martensill Karsch*
bp	base pair
BSA	bovine serum albumin
cDNA	complementary DNA
Da	Dalton
DMSO	dimethyl sulphoxide
DNA	deoxyribonucleic acid
dNTP	deoxynucleotide triphosphate
ED_{50}	paralytic unit
EDTA	ethylene diaminetetra-acetic acid
ELISA	enzyme linked immunoassay
Fab	fragment antigen binding
FBS	fetal bovine serum
Fc	fragment constant
GM	midgut membrane
GST	glutathione S-transferase
HAT	hypoxanthine, aminopterin, thymidine
HPLC	high pressure liquid chromatography
HPRT	hypoxanthine phosphoribosyl transferase enzyme
HT	holocyclus toxin
HWTX	Huwentoxin
Ig	immunoglobulin
IMAC	immobilized metal affinity column

IPTG	isopropylthio-β-D-galactopyranoside
kbp	kilobasepairs
kDa	kiloDalton
KTX2	kaliotoxin2
LB	Luria broth
LD_{50}	dose causing 50% death
LqhαIT	*Leiurus quinquestriatus hebraeus* alpha insect toxin
mA	milliamps
mAb	monoclonal antibody
MBP	maltose binding protein
mM	millimolar
MW	molecular weight
MWCO	molecular weight cut off
NaAz	sodium azide
NBT	nitro blue tetrazolium
Ni-NTA	nickel-nitrilotriacetic acid
OD	optical density
PCR	polymerase chain reaction
pmol	picomole
p-NPP	p-nitrophenyl phosphate
PVDF	polyvinylidinedifluoride
rpm	revolutions per minute
SDS-PAGE	sodium dodecyl sulphate-polyacrylamide gel electrophoresis
SSNAP	sparingly soluble non-antigenic protein
%T	total acrylamide concentration (w/v)
TBE	tris buffered ethylene diaminetetra-acetic acid
TEMED	N,N,N',N'-tetramethylenediamine
TFA	trifluoroacetic acid
TsNTxP	*Tityus serrulatus* nontoxic protein
U	unit

UCH-L1	ubiquitin carboxyl terminal hydrolase-L1
USP	ubiquitin specific protease
UV	ultraviolet
V	volts
YUH1	yeast deubiquitinating hydrolase 1

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ABSTRACT

Acute, ascending, flaccid motor paralysis of forelimbs and death due to respiratory failure is the dominant characteristic of tick toxicosis by the Australian paralysis tick, *Ixodes holocyclus*. A tick toxin vaccine has the potential to be an effective preventative measure against tick toxicosis that affects thousands of domestic and companion animals each year. In previous research for the development of an anti-tick vaccine, Masina (1999) used a maltose-binding protein (MBP) holocyclus toxin (HT-1) fusion protein as immunogen. It was partially protective against challenges with crude tick extract in neonatal mice but unable to protect dogs against paralysis caused by direct tick attachment.

Subsequently, four monoclonal antibodies generated in this study against the fusion protein were found to be incapable of binding to the native toxin in crude tick extract in Western blots. These results indicated that the HT-1 component of the MBP fusion protein was incorrectly folded compared to the native toxin.

An expression system using ubiquitin fusion protein was investigated. Ubiquitin is a 10 kDa carrier protein which is smaller than the 43 kDa MBP. A smaller fusion partner was considered as it is less likely to interfere with HT-1 folding. The ubiquitin-HT-1 fusion protein was expressed in both soluble and insoluble forms, corresponding to the cytoplasmic fraction and inclusion bodies. The soluble form could be purified under non-denaturing conditions utilising the incorporated His-6-tag and a Ni affinity column. The insoluble form, like the periplasmically expressed MBP-HT-1 fusion protein, could only be purified using denaturing conditions.

The purified soluble ubiquitin-HT-1 fusion protein appeared to have the correct conformational folding as it was recognized by commercial dog anti-tick serum in Western blots. The purified soluble ubiquitin-HT-1 fusion protein was used to immunise rabbits and mice for protection experiments, but was found to be unprotective. However, serum from immunized animals was able to detect a 5 kDa protein from crude tick extract in a Western blot. This 5 kDa protein was also recognized by the commercial dog anti-tick serum and

has the molecular weight corresponding to the HT-1 neurotoxin. Creation of monoclonal antibodies to this ubiquitin-HT-1 fusion protein may therefore aid future development towards a tick vaccine. These antibodies could in turn be used to isolate native HT-1 from the crude tick extract instead of a combination of conventional chromatography methods. This approach would allow isolation of HT-1 in more significant quantities than is currently possible to enable confirmation of the HT-1 sequence. Suspected problems associated with incorrect sequence or interference by the carrier protein to cause a non-protective response could also be resolved.